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Substance Group: **Group 4: Dithiophosphate Alkyl Esters**

Summary prepared by: **Petroleum Additives Panel**
 Health & Environmental Research Task Group

Date of last update: **December 2005**

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1.0 Physical Chemical Properties

1.1 Water Solubility

CAS No.	CAS# 84605-28-7
Test Substance Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters
Method/Guideline	Flask Method, Method A6 of Commission Directive 92/69/EEC and OECD Method 105
GLP (Y/N)	Yes
Year	2004
Remarks for Test Conditions	<p>The solubility in water of a substance is specified by the saturation mass concentration of the substance in water at a given temperature. Preliminary tests were conducted by diluting 0.4934 and 2.0138 g of the test material to 100 mL with glass double-distilled water. After shaking at 30°C for 6 or 5 hours and standing at 20°C for 18 or 21 hours, the solutions were centrifuged at 13,500 rpm for 15 minutes and analyzed.</p> <p>Based on the preliminary results, mixtures of double distilled water and test substance were added to each of six conical flasks. Two flasks each were shaken for approximately 24, 48, and 72 hours at 30°C. After standing at 20°C for not less than 24 hours, the contents of each flask was centrifuged at 13500 rpm for 15minutes and the supernatant transferred into glass bottles. The concentration of the test material in the sample solutions was determined by high performance liquid chromatography with mass selective detection.</p>
Results	<p>Prior to sampling the solutions were slightly cloudy with excess test material present. After centrifugation the samples were clear, colorless and visually free from excess test material.</p> <p>The water solubility of the test material was determined to be 19.6 g/l at 20.0 ±0.5°C.</p> <p>The linearity of the detector response in respect to concentration was acceptable over a range of concentrations of 0 to 1 mg/L with a correlation coefficient of 1.00.</p>
Value (g/L) at temperature °C	19.6 g/l at 20.0 ±0.5°C.
Conclusions	The water solubility of the test material was determined to be 19.6 g/l at 20.0 ±0.5°C.
Data Quality	Reliable without restriction (Klimish Code).
References	Determination of Water Solubility SafePharm Laboratories Project No.: 1666/041 (10 March 2005)

1.2 Biodegradation

Robust Summary 4-Biodeg-1

Test Substance	
CAS #	84605-29-8
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters, zinc salts
Remarks	This substance is referred to as Mixed isopropyl and 1, 3-dimethylbutyl derivative in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 1.1 "Identity and Chemistry of Zinc Dialkyl dithiophosphates" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD Method 310B; & EEC Method C.4-C (2).
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	<p><u>Inoculum</u>: Activated sludge from domestic waste water treatment plant was sieved through a 2 mm screen, aerated for 4 hours and homogenized in a blender. The sludge was allowed to settle for 30 mins and supernatant was used as the inoculum the same day that it was prepared.</p> <p><u>Concentration of test chemical</u>: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks.</p> <p><u>Temp of incubation</u>: $20 \pm 2^{\circ}\text{C}$.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume.</p> <p><u>Test Setup</u>: Total volume of liquid in test chambers was 3 L. The biodegradation test was started by bubbling CO₂ free air through the test media at a rate of 50 to 100 mL per minute. The CO₂ generated within each test chamber was trapped as K₂CO₃ in the KOH solution and measured using a carbon analyzer.</p> <p><u>Sampling frequency</u>: CO₂ traps were removed for analysis on Days 1, 4, 8, 11, 14, 19, 21, 25, and 29. The CO₂ trap nearest the chamber was removed. On day 28, the test was terminated by the acidification of the</p>

	<p>test chamber and aerated overnight to release dissolved CO₂. The trapping solutions closest to the test chambers were analyzed for inorganic carbon.</p> <p><u>Controls</u>: Blank and positive controls were included; abiotic and toxicity controls were not. Sodium benzoate was used as the reference substance in the positive controls.</p> <p><u>Analytical method</u>: KOH ("trap") solutions were used downstream of the test flasks to trap generated CO₂ as K₂CO₃. The CO₂ produced was measured using a carbon analyzer.</p> <p><u>Method of calculating measured concentrations</u>: N/A</p> <p><u>Other</u>: n/a</p>
<u>Results</u>	
Test Substance Degradation, % after time	5.9% after 28 days
Kinetic (for sample, positive and negative controls)	<p>Reference (Sodium benzoate): 99.3%. An average percent biodegradation of 60% was achieved within 7 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14.</p> <p>Test substance: 1.5% (28d)</p>
Breakdown Products (Y/N) If yes describe breakdown products	N
<u>Remarks</u>	
<u>Conclusions</u>	Test substance degraded 1.5% in 28 days. The reference substance, canola oil, degraded 99.3% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

Robust Summary 4-Biodeg-2

<u>Test Substance</u>	
CAS #	54261-67-5
Chemical Name	Phenol, dodecyl-, hydrogen phosphorodithioate zinc salt
Remarks	This substance is referred to as dodecyl derivative in the HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category. For more information on the chemical, see Section 1.1 "Identity and Chemistry of Zinc Dialkyldithiophosphates" in HERTG's Test Plan for Zinc Alkyl Dithiophosphate Category.
<u>Method</u>	
Method/Guideline followed	OECD Method 310B; U.S. EPA Method 796.3260; ASTM D5864-95.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	N
Year (Study Performed)	1998
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	<p><u>Inoculum</u>: Soil was collected from a wooded lot to a depth of 20 cm (surface soil was not included). Prior to use, 200 gm (wet weight) of soil was suspended in 2 L of water, allowed to settle for 30 min and filtered through glass wool. Filtrate was aerated until use. Activated sludge from domestic wastewater treatment plant was sieved through a 2 mm screen, aerated for 4 hours and homogenized in a blender. The sludge was allowed to settle for 30 minutes and supernatant was removed for use.</p> <p><u>Concentration of test chemical</u>: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks.</p> <p><u>Temp of incubation</u>: $20 \pm 3^{\circ}\text{C}$.</p> <p><u>Dosing procedure</u>: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume.</p> <p><u>Test Setup</u>: Total volume of liquid in test chambers was 3 L. The biodegradation test was started by bubbling CO₂ free air through the test media at a rate of 50 to 100 mL per minute. The CO₂ generated within each test chamber was trapped as K₂CO₃ in the KOH solution and measured using a carbon analyzer.</p> <p><u>Sampling frequency</u>: CO₂ traps were removed for analysis on Days 2, 5, 11, 13, 16, 18, 23, and 29. On day 28, the test was terminated by the acidification of the test chamber to release dissolved CO₂.</p>

	<p><u>Controls:</u> Blank and positive controls were included; abiotic and toxicity controls were not. Canola oil was used as the reference substance in the positive controls.</p> <p><u>Analytical method:</u> KOH ("trap") solutions were used downstream of the test flasks to trap generated CO₂ as K₂CO₃. The CO₂ produced was measured using a carbon analyzer.</p> <p><u>Method of calculating measured concentrations:</u> N/A</p> <p><u>Other:</u> A preadapted inoculum was used for the biodegradation test. Equal volumes of the activated sludge supernatant and soil filtrate were combined and supplemented with vitamin free casamino acids and 25 mg/L yeast extract. 100 ml of the supplemented inoculum was combined with 900 ml of test medium within each 2-L erlenmeyer flask. The solutions were aerated with CO₂ free air and test substances added incrementally at concentrations equivalent to 4, 8, and 8 mg C/L on days 0, 7, and 11, respectively. On day 14, an equal volume of each culture was combined and composite inoculum screened using glass wool and homogenized in a blender. Standard plate count prior to the 14-day adaptation period was 1.36×10^5 CFU/mL. Standard plate count after 14-day adaptation was 2.62×10^5 CFU/mL.</p>
<u>Results</u>	
Test Substance Degradation, % after time	5.9% after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Canola oil): 92% (2 - 5 day lag period) Test substance: 5.9% (28d; 11 – 16 day lag period)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
<u>Conclusions</u>	Test substance degraded 5.9% in 28 days. The reference substance, canola oil, degraded 92% in the same test period.
<u>Data Quality</u>	(1) Reliable without restrictions.
<u>References</u>	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
<u>Other</u>	Updated: 11-19-2000

1.3 Hydrolysis

Test Substance*:	CAS No. 5810-88-8; Phosphorodithioic acid, 0,0-bis(2-ethylhexyl) esters
Method/Guideline:	None Cited
Year (guideline):	Not applicable
Type (test type):	Hydrolytic evaluation
GLP:	No
Year (study performed):	1993
Estimated pH:	7
Test Conditions: (FT - TC)	The dithiophosphate ester was combined with 20% water at: a) Room temperature for 1 hour. b) 80 C for 1 hour.
Test Results: (FT - RS)	Hydrolytic species were determined using NMR. The results indicate that there is no significant hydrolysis at room temperature. At 80 C, about 50% of the thioacid is hydrolyzed to the phosphorothioic acid ester and hydrogen sulfide. Continued heating at high temperature results in the formation of the mono ester and eventually, the phosphorothioic acid itself.
	<p>More information on the dithiophosphate esters can be found in the test plan for this category prepared by the American Chemistry Council, Petroleum Additives Panel - Health, Environmental, Regulatory, Task Group. (1)</p> <p>1. Petroleum Additives Panel - Health, Environmental, Regulatory, Task Group. 2002 Category Analysis Document and Testing Plan For Dithiophosphate Esters Category. American Chemistry Council, Petroleum Additives Panel - Health, Environmental, Regulatory, Task Group. VA, USA.</p>
Test Substance: (FT - TS)	<p>CAS# 5810-88-8; Phosphorodithioic acid, 0,0-bis(2-ethylhexyl) esters</p> <p>Eight related substances are included in the Dithiophosphate Esters category:</p> <ul style="list-style-type: none"> Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and isopropyl) esters – (CAS # 84605-28-7), referred to as “mixed 1,3-

	<p>dimethylbutyl and iso-propyl derivative”</p> <ul style="list-style-type: none"> • Phosphorodithioic acid, mixed O,O-bis(iso-butyl and pentyl) esters – (CAS # 68516-01-8), referred to as “mixed isobutyl and pentyl derivative” • Phosphorodithioic acid, mixed O,O-bis(sec-butyl and 1,3 dimethylbutyl) esters – (CAS # 68784-30-5), referred to as “mixed sec-butyl and 1,3-dimethylbutyl derivative” • Phosphorodithioic acid mixed O,O-bis(sec-butyl and isooctyl) mixed esters – (CAS # 113706-14-2), referred to as “mixed sec-butyl and isooctyl derivative” • Phosphorodithioic acid, mixed 0,0-bis(2-ethylhexyl and iso-butyl) esters – (CAS # 68784-32-7), referred to as “mixed 2-ethyl hexyl and isobutyl derivative” • 2-Pentanol, 4-methyl-hydrogen phosphorodithioate – (CAS # 6028-47-3), referred to as “1,3-dimethylbutyl derivative” • Phosphorodithioic acid, 0,0-bis(2-ethylhexyl) esters – (CAS# 5810-88-8), referred to as “2-ethylhexyl derivative” • Phosphorodithioic acid, O,O-diisooctyl ester – (CAS# 26999-29-1), referred to as “isooctyl derivative” <p>Dithiophosphate Esters consist of a phosphorodithioic acid structure with alkyl ester substituent groups. The alkyl groups are saturated hydrocarbon chains that vary in length and extent of branching.</p> <p>These substances are prepared by reacting phosphorous pentasulfide with one or more primary or secondary alcohols to form the phosphorodithioic acid ester. The reaction produces hydrogen sulfide as a by-product, which is removed by sparging the material with nitrogen. The material is then filtered to remove residual phosphorus pentasulfide and stored as a closed system intermediate. These esters are not transported to other sites, but are converted to the commercial zinc diaklydithiophosphates in a subsequent step at the same manufacturing site.</p> <p>The alkyl ester substituent group may vary in chain length (e.g., C3 to C8) and in the degree of branching. Although branched alkyl groups are generally presumed to be more water-soluble than straight chains, Dithiophosphate Esters have such low water solubility that the degree of branching does not materially affect their solubility and performance in petroleum base stocks.</p>
Conclusion: (FT - CL)	Based on NMR analysis, products in this category do not hydrolyze at room temperature at a pH of 7. Hydrolysis only occurs at elevated temperatures.
Reliability: (FT - RL)	(2) Reliable with restrictions

	The data include analysis at one pH at two temperatures and represent the potential for hydrolysis for the products with the 8 CAS numbers listed under test substance.
Reference: (FT - RE)	American Chemistry Council; Petroleum Additives Panel; Health, Environmental, Regulatory, Task Group
Other (source): (FT - SO)	Not Applicable

2.0 Aquatic Toxicity

2.1 Acute Toxicity to Fish

<u>Test Substance</u>	
CAS #	84605-28-7
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Dithiophosphate Alkyl Esters Category.
<u>Method</u>	
Method/Guideline followed	OECD 203
Test Type	Acute Toxicity to Fish (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Rainbow Trout (<i>Oncorhynchus mykiss</i>) Fingerlings were obtained from a commercial breeder.
Fish Size	Average length 4.4 cm and a mean weight of 1.16 g, loading rate of 0.58 g body weight/L
Number of Fish	Range Find: 3/concentration Definitive Study 20/concentration (10/replicate)
Analytical Monitoring	No
Nominal Test Substance Concentration Levels	Range Find Study: 0, 10, 100 mg/L WAF Definitive Study: 0, 100 mg/L WAF
Test Concentration Preparation	A measured weight of test material was added to a measured volume of dechlorinated tap water and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer. Mixing speed was adjusted such that a slight vortex formed causing a slight dimple at the surface. Following the mixing period, the test solution was allowed to stand for one hour. A small amount of the WAF was removed and examined microscopically for the presence of micro-dispersions or globules of test material. The mid-depth siphoned phase (i.e., WAF) was used for the aquatic toxicity test. The preparation was conducted in duplicate for replicates one and two.
Exposure Period	96 hours
Exposure Conditions	Static-renewal test conditions. The test preparations were renewed daily.
Vehicle	None
Statistical Analysis	LL ₅₀ values determined by inspection.
Dose Range finding Study	Yes
Test Chambers	Covered, 20-liter glass aquaria containing the test solution
Diluent Water	Dechlorinated, softened tap water
Diluent Water Chemistry	Hardness 100 mg/l as CaCO ₃
Diluent Water Chemistry During 96 Hour Exposure	Dissolved Oxygen: 9.1-10.3 mg/L pH: 6.8-8.1

Period.	
Photoperiod	16 hours of light, 8 hours of dark
Temperature Range	Approximately 14°C during holding period 13.9-14.7°C during exposure period
Remarks field for test conditions	All organisms were observed for mortality and clinical signs of toxicity or abnormal behavior at 3, 6, 24, 48, 72, and 96 hours after initiation of test material exposure.
<u>Results</u>	<p>At the start of the mixing period, the 100 mg/L loading rate was a clear, colorless water column with gray globules of test material throughout. At the end of the mixing period, the 100 mg/L loading rate was a clear, colorless water column with black globules of test material throughout. After the 1 hour settling period the 100 mg/L loading rate was a clear, colorless water column with globules of test material at the surface and bottom of the vessel. Microscopic examination of the WAF indicated no micro-dispersion or undissolved test material to be present in the water column.</p> <p>No mortality was observed in the range find or definitive studies. No sub-lethal effects were observed at the 100 mg/L WAF.</p> <p>The 96-hour Lethal Loading rate (LL₅₀) was >100 mg/L (WAF). The No Observed Effect Level, based on the lack of mortality and the absence of any sub lethal effects of exposure was 100 mg/L.</p>
<u>Conclusions</u>	Under the conditions of this study the 96-hour Lethal Loading rate (LL ₅₀) was >100 mg/L (WAF). The 96-hour no observed effect level was 100 mg/L (WAF).
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Acute Toxicity to Rainbow Trout SafePharm Laboratories Project No.: 1666/070 (19 SEP 2005)
<u>Other</u>	Updated: 10/3/2005

2.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Test Substance	
CAS #	84605-28-7
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Dithiophosphate Alkyl Esters Category.
Method	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #202 Daphnia sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	2004
Species/Strain	Daphnia magna
Analytical Monitoring	Test material concentrations of exposure solutions were not determined.
Exposure Period (unit)	48 hours
Statistical methods	EL50 values calculated using the trimmed Spearman-Kärber method (ToxCalc software 1999).
Remarks field for test conditions (fill as applicable)	<p>Twenty-four hours old Daphnia magna derived from in house cultures were used for the study. Individual water accommodated fractions (WAFs) were prepared for each test level. Appropriate amounts of test material was added to a measured volume of reconstituted water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer at a stirring rate that produced a slight dimple at the surface of the water. Following mixing period, the test solutions were allowed to stand for 1.5 hours before the water phase was gently siphoned (first 75-100 mL discarded) from the mixing vessel by mid-depth siphoning into the test vessels.</p> <p>The test chambers were covered, 250 ml vessels that contained 200 ml of test solution. Ten daphnids/time point were distributed into each concentration for the range finding study. Ten daphnids/replicate/time point (2 replicates) were used in the definitive study. Test vessels were covered to reduce evaporation and were maintained at 20.5 to 21.3°C with a photoperiod of 16 hours light and 8 hours dark. Daphnia were not fed nor were cultures aerated during exposure. Control groups were handled in the same manner as the test groups. Test preparations were not renewed during the exposure period.</p> <p>Water temperature was recorded daily throughout the test. Dissolved oxygen concentration and pH were recorded at the start and end of the study.</p> <p>Any immobilization or adverse reactions to exposure were recorded at 24 and 48 hours after the start of exposure. Daphnia were considered immobilized if they were unable to swim for approximately 15 seconds after gentle agitation.</p>
Test Concentrations	<p>Range Find Study: 0, 10, 100 mg/L WAF</p> <p>Definitive Study: 0, 10, 18, 32, 56 and 100 mg/L WAF</p>
Results	The 48-hour EL50 (Effective Loading rate) was determined to be 36 mg/L (WAF) (95% Confidence limits 32-40 mg/L).
Remarks	<p>Temperature was maintained at approximately 21°C throughout the test. No treatment related differences were observed in oxygen concentration.</p> <p>Concentration dependent differences in pH were observed throughout the</p>

	<p>study.</p> <p>After 24 hours stirring and a 1.5-hour standing period the 10, 18, 32, 56 and 100 mg/L loading rates were colorless with an oily slick of test material floating at the water surface. After siphoning and for the duration of the test, the loading rates were clear colorless solutions. No micro-dispersions or undissolved test material were present upon microscopic examination.</p> <p>Cumulative immobilization data was as follows:</p> <table><tr><th colspan="4">Cumulative Immobilization (%)</th></tr><tr><th>Concentration mg/L (WAF)</th><th>Number of Daphnia</th><th>24 Hours</th><th>48 Hours</th></tr><tr><td colspan="4">Range Find</td></tr><tr><td>0</td><td>10/interval</td><td>0</td><td>0</td></tr><tr><td>10</td><td>10/interval</td><td>0</td><td>0</td></tr><tr><td>100</td><td>10/interval</td><td>100</td><td>100</td></tr><tr><td colspan="4">Definitive Study</td></tr><tr><td>0</td><td>20/interval</td><td>0</td><td>0</td></tr><tr><td>10</td><td>20/interval</td><td>0</td><td>0</td></tr><tr><td>18</td><td>20/interval</td><td>0</td><td>0</td></tr><tr><td>32</td><td>20/interval</td><td>0</td><td>30</td></tr><tr><td>56</td><td>20/interval</td><td>0</td><td>100</td></tr><tr><td>100</td><td>20/interval</td><td>70</td><td>100</td></tr></table> <p>The 48-hour EL50 (Effective Loading rate) was determined to be 36 mg/L (WAF) (95% Confidence limits 32- 40 mg/L).</p> <p>The no observed effect-loading rate at 48-hours was 18 mg/L (WAF).</p>	Cumulative Immobilization (%)				Concentration mg/L (WAF)	Number of Daphnia	24 Hours	48 Hours	Range Find				0	10/interval	0	0	10	10/interval	0	0	100	10/interval	100	100	Definitive Study				0	20/interval	0	0	10	20/interval	0	0	18	20/interval	0	0	32	20/interval	0	30	56	20/interval	0	100	100	20/interval	70	100
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Data Quality	Reliable without restriction (Klimisch Code).																																																				
References	Unpublished confidential business information SafePharm Laboratories Project No.: 1666/069																																																				
Other	Updated: 3/22/2005																																																				

2.3 Toxicity to Aquatic Plants (e.g. Algae)

<u>Test Substance</u>	
CAS #	84605-28-7
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Dithiophosphate Alkyl Esters Category.
<u>Method</u>	
Method/Guideline followed	OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test (Water Accommodated Fraction-WAF)
GLP (Y/N)	Y
Year (Study Performed)	2005
Species/Strain	Freshwater algae, <i>Scenedesmus subspicatus</i> /CCAP 276/20
Element basis (# of cells/mL)	Approximately 1.90×10^6 cells/mL, 2.5 mL used to inoculate 500 mL of medium for an initial cell density of 10^4 cells/mL.
Exposure period/duration	72 hours
Range find test	Yes
Analytical monitoring	Not performed
Statistical methods	One-way analysis of variance incorporating Bartlett's test for homogeneity of variance and Dunnett's multiple comparison procedure were used to compare the area under the growth curve data of the treated and control groups at 72 hours.
Remarks field for test conditions (fill as applicable)	<p>Test Species: Cultures obtained from the Culture Collection of Algae and Protozoa (CCAP), Institute of Freshwater Ecology, The Ferry House, Far Sawrey, Ambleside, Cumbria, U.K.</p> <p>Loading Concentration: Range Find Study: 0, 10, 100 and 1000 mg/L (WAF) Definitive Study: 0, 6.25, 12.5, 25, 50 and 100 mg/L (WAF)</p> <p>Test System: A measured weight of test material was added to a measured volume of culture media in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stirrer. Mixing speed was adjusted such that a slight vortex formed causing a slight dimple at the media surface. Following the mixing period, the test solution was allowed to stand for one hour. A small amount of the WAF was removed and examined microscopically for the presence of micro-dispersions or globules of test material. Microscopic examination of the WAF confirmed that there were no micro-dispersions of test material present. The siphoned phase (i.e., WAF) was used for the aquatic toxicity test.</p> <p>Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. Two (range find study) or three (definitive study) 100-mL replicates per treatment, inoculum $\sim 10,000$ cells/mL. The 250-mL conical flasks were plugged with polyurethane foam bungs. During the test all</p>

treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 150 cycles per minute under constant light (24 hours/day) for

	<p>72 hours. Cell densities were determined using a Coulter Multisizer Particle Counter at 0, 24, 48 and 72 hours. pH was determined at 0 and 72 hours.</p> <p>Light: Continuous illumination approximately 7000 lux.</p> <p>Test temperature: 24.0° C.</p> <p>Culture Media: As specified in the guideline.</p> <p>Method of calculating mean measured concentrations: Not applicable.</p> <p>Exposure period: 72 hours</p>
<u>Results</u>	<p>Range Find Study: No effect on growth at 10 mg/L WAF. Growth reduced at 100 and 1000 mg/L WAFs.</p> <p>Definitive Study: Both growth and biomass were affected by the presence of the test material over a 72 hour period.</p> <p>The E_bL_{50}, the loading rate that reduced biomass by 50%, was 55 mg/L WAF. The E_rL_{50}, the loading rate that reduced specific growth by 50%, was 87 mg/L WAF.</p> <p>The No Observed Effect Loading Rate (NOEL) was 25 mg/L WAF.</p> <p>The cell concentrations of the control cultures increased by a factor of 56 during the study meeting the guideline requirement of at least a factor of 16 after 72 hours.</p> <p>All test and control cultures were inspected microscopically at 72 hours. No abnormalities were observed in the control or treated cultures at 6.25, 12.5, 25 or 50 mg/L WAFs. Few intact cells were observed in the cultures at the 100 mg/L WAF.</p> <p>At the start of the test all control and test cultures were clear colorless solutions. At 72 hours the control and treated cultures at concentrations up to 50 mg/L WAF were green dispersions. The cultures at 100 mg/L WAF were clear colorless solutions.</p> <p>Control culture pH increased from 7.2 at 0 hour to 8.4-8.5 at 72 hours. At both 0 and 72 hours the test preparations showed a concentration dependent decrease in pH with the lowest concentrations exhibiting the highest pH. This was attributed to the presence of the test material.</p>
<u>Conclusions</u>	<p>Both the growth and the biomass of <i>Scenedesmus subspicatus</i> (CCAP 276/20) were affected by the presence of the test material over the 72-hour exposure period.</p> <p>The E_bL_{50}, the loading rate that reduced biomass by 50% was 55 mg/L WAF. The E_rL_{50}, the loading rate that reduced specific growth by 50%, was 87 mg/L WAF.</p>

	The No Observed Effect Loading Rate (NOEL) was 25 mg/L WAF.
<u>Data Quality</u>	(1) Reliable without restriction
<u>References</u>	Algal Inhibition Test SafePharm Laboratories Project No.: 1666/068 (21 SEP 2005)
<u>Other</u>	Updated: 10/3/2005

3.0 Mammalian Toxicity

3.1 Acute Toxicity

3.1.1 Acute Dermal Toxicity

<u>Test Substance</u>	
CAS #	Mixed Isobutyl, isooctyl and pentyl derivative
Chemical Name	Phosphorodithioic acid mixed O,O-bis(iso-Bu and isooctyl and pentyl) esters
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Dithiophosphate Alkyl Esters Category.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1984
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex/group	5
Vehicle	None
Route of administration	Dermal
Dose level	2 g/kg
Dose volume	2 ml/kg
Control group included	No
Remarks field for test conditions	<p>This study deviates from the above referenced guideline in that the dosing site was abraded prior to treatment. This was not considered a significant deviation from the guideline.</p> <p>Approximately 24 hours prior to topical application of the test material, the hair of each animal was closely clipped. Immediately prior to dosing the skin was abraded. A single dose of 2 g/kg of the undiluted test material was administered dermally to five male and female animals. The test material was kept in contact with the skin for a period of 24 consecutive hours under a gauze and elastic bandage. The application site was wiped clean of residual test material at the end of the 24-hour exposure period. The animals were observed for abnormal clinical signs frequently on the day of dosing and once daily for 14 days after treatment. Individual body weights were recorded on the day of dosing. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals on Day 14.</p>

<u>Results</u>	LD50 > 2.0 g/kg (males and females)
Remarks	No mortality was observed. Clinical signs observed in all animals included cyanosis and decreased motor activity. The majority of animals exhibited motor incoordination. Four animals exhibited a loss of righting reflex. Recovery from most of these signs occurred by day three post treatment. Dermal findings included necrosis, edema and ulceration. Dermal irritation persisted through study termination. Gross pathological findings were limited to pitted kidneys in one female.
<u>Conclusions</u>	The test article, when administered dermally as received to 5 male and 5 female New Zealand white rabbits had an acute dermal LD50 of greater than 2.0 g/kg.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/13/00 (RTA-048)

3.1.2 Acute Inhalation Toxicity

<u>Test Substance</u>	
CAS #	Mixed Isobutyl, isooctyl and pentyl derivative
Chemical Name	Phosphorodithioic acid mixed O,O-bis(iso-Bu, and isooctyl and pentyl) esters
Purity	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Dithiophosphate Alkyl Esters Category.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 403
Test Type	Acute Inhalation toxicity (Limit Test)
GLP (Y/N)	N
Year (Study Performed)	1986
Species/Strain	Rats/Sprague-Dawley
Sex	Male and female
No. of animals/sex	5
Vehicle	None
Route of administration	Vapor inhalation (single 4 hour whole body exposure)
Dose level	0.198 mg/L which included 74.2 ppm H ₂ S (actual maximum attainable concentration)
Vehicle control group	No
Chamber analysis	Yes (for hydrogen sulfide)
Remarks field for test conditions	One group of five rats/sex was exposed for 4 hours to the test material as a vapor generated by a glass distillation column filled with glass beads and heated to approximately 100°C. The distillation column was attached to a 3-neck flask. Test material was pumped into the top of the column. A portion of test material was vaporized and generated an atmosphere containing H ₂ S, among other possible vapors. The vapor was delivered into a 70-liter glass exposure chamber. The actual exposure concentration of H ₂ S as measured by gas chromatography was 74.2 ppm. The nominal concentration of the test material in the atmosphere was 0.198 mg/L. Animal observations for toxicological signs and mortality were recorded periodically during exposure and at least once daily during the 14 day observation period. Individual body weights were recorded on Day1 (immediately prior to exposure) and on Days 7 and 14. Serum cholinesterase evaluations were performed on all animals approximately 18 hours before and 30 minutes following exposure. Animals were sacrificed and subjected to a complete gross necropsy following the 14-day observation period.
<u>Results</u>	LC50 > 0.198 mg/L which included 74.2 ppm H ₂ S (maximum attainable concentration)
Remarks	All animals survived the exposure and observation periods. Lacrimation was recorded in two animals during exposure. Salivation, redness around the nose and discoloration around the mouth were

	observed following exposure. All animals appeared normal by day 8. All animals gained weight during the two-week study period. Serum cholinesterase values were variable and did not exhibit a consistent effect of test material exposure. One female exhibited gray lungs at necropsy. There were no abnormal postmortem findings evident in any of the other animals at study termination.
<u>Conclusions</u>	Following 4-hour whole body exposure to the test material vapor the LC50 in male and female Sprague Dawley rats was >0.198 mg/L which included 74.2 ppm H ₂ S. This was the maximum concentration attainable.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Unpublished confidential business information
<u>Other</u>	Updated: 7/1400 (RTA-050)

3.2 Genetic toxicity

Robust Summary 4-Gentox-1

<u>Test Substance</u>	
CAS #	CAS# 84605-28-7
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters
Remarks	For more information on this chemical, see Section 2.0 “General Substance Information” in HERTG’s Final Submission for Dithiophosphate Alkyl Esters Category.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	2005
Test System	<i>Salmonella typhimurium</i>
Strains Tested	<i>Salmonella typhimurium</i> tester strains TA98, TA100, TA102, TA1535, TA1537
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0, 50, 150, 500, 1500, 5000 µg/plate
Metabolic Activation	With and without (0.5 mL of S9 fraction mix of livers of phenobarbitone/beta-naphthoflavone pretreated Sprague Dawley rats. Before use each batch of S9 was assayed for its ability to metabolize appropriate indirect mutagens used in the Ames assay. S9 was stored at –196°C.
Vehicle	Dimethyl sulphoxide
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 5.0 ug/plate TA98 -S9 4-nitroquinoline-1-oxide 0.2 ug/plate TA100 +S9 2-aminoanthracene 1.0 ug/plate TA100 -S9 N-ethyl-N’-nitro-N-nitrosoquandine 3.0 ug/plate TA102 +S9 1,8-dihydroxyanthraquinone 10.0 ug/plate TA102 -S9 mitomycin C 0.5 ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 N-ethyl-N’-nitro-N-nitrosoquandine 5.0 ug/plate TA1537 +S9 2-aminoanthracene 2.0 ug/plate TA1537 -S9 9-aminoacridine 80.0 ug/plate
Vehicle Control	Dimethyl sulphoxide
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strain TA100, and dose levels of test material

	ranging from 0.05 to 5000 µg/plate.
Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 0.1 mL of test material, positive control or vehicle control, 0.1 mL of tester strain, 0.5 mL of S9 mix (if needed) or phosphate buffer were mixed in a test tube and added to the surface of a minimal agar plate. Plates were incubated for 48 hours at 37°C. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. The frequency of revertant colonies was assessed using a Domino colony counter.
<u>Results</u>	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	<p>No cytotoxicity was observed in the dose rangefinding study with tester strain TA100 with or without metabolic activation as evidenced by normal background lawn and no reduction in the number of revertants/plate.</p> <p>In the mutation assays the test material caused no visible reduction in the growth of the bacterial lawn at any dose level. An oily precipitate was observed at and above 1500 µg/plate, this did not prevent the scoring of plates. No significant increases in the frequency of revertant colonies were recorded for any of the tester strains, at any dose level either with or without metabolic activation.</p> <p>The positive control for each respective test strain induced marked increases (with or without S9) over the mean value of the vehicle control for a given strain, confirming the expected positive control response.</p>
<u>Conclusions</u>	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Reverse Mutation Assay "Ames Test" Using Salmonella Typhimurium Safepharm Study Number: 1666/0077 (13 SEP 2005)
<u>Other</u>	Updated: 9/27/2005

Robust Summary 4-Gentox-2

<u>Test Substance</u>	
CAS #	CAS# 84605-28-7
Chemical Name	Phosphorodithioic acid, mixed O,O-bis (1,3-dimethylbutyl and iso-Pr) esters
Remarks	For more information on this chemical, see Section 2.0 "General Substance Information" in HERTG's Final Submission for Dithiophosphate Alkyl Esters Category.
<u>Method</u>	
Method/Guideline followed	OECD Guideline 473
Test Type	<i>In Vitro</i> Chromosomal Aberration Assay
GLP (Y/N)	Y
Year (Study Performed)	2005
Test System	Human peripheral blood lymphocytes
Exposure Method	Dilution
Test Substance concentration levels	<p>Experiment 1</p> <p>4 hour treatment, 20 hour harvest without activation: 0*, 78.13, 156.25, 312.5*, 468.75*, 625*, 937.5* µg/mL</p> <p>4 hour treatment, 20 hour harvest with activation (2% S9): 0*, 39.06, 78.13, 156.25*, 312.5*, 468.75*, 625*µg/mL</p> <p>Experiment 2</p> <p>24 hour treatment, 0 hour harvest without activation: 0*, 9.77, 19.53*, 39.06*, 58.6*, 78.13, 156.25 µg/mL</p> <p>4 hour treatment, 20 hour harvest with activation (1% S9): 0*, 78.13, 156.25*, 312.5*, 625*, 937.5, 1250 µg/mL</p> <p>* = Dose levels selected for metaphase analysis.</p>
Metabolic Activation	With and without S9 fraction mix of livers of phenobarbitone and beta-naphthoflavone pretreated Sprague Dawley rats
Vehicle	Acetone
Vehicle and Positive Control concentration levels by activation status	<p>Mitomycin C - non-activated test system positive control (0.2 µg/mL)</p> <p>Cyclophosphamide - activated test system positive control (5 µg/mL)</p> <p>Acetone – solvent control</p>
Statistical Analysis	Positive control groups were compared to vehicle control by Fisher Exact Test. Differences between control and treated groups were compared using Fisher Exact Test.
Preliminary Toxicity Dose Range Finding Assay	Consisted of an evaluation of test article effect on mitotic index. Evaluation performed at 4 hours with and without activation followed by a 20-hour recovery period and following a continuous 24 hour exposure without metabolic activation. Concentrations of test material evaluated ranged from 19.53 to 5000 µg/mL.
Remarks field for test	A pretest dose range finding study was conducted at concentrations up to 5000

conditions	<p>µg/mL with and without metabolic activation. In the main study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. Mitomycin C (positive control) was tested without activation and Cyclophosphamide (positive control) was tested with activation. Prepared cultures were treated with test substance or control material. Two experiments were conducted as follows:</p> <p>Experiment 1 4 hour treatment, 20 hour harvest without activation: 0*, 78.13, 156.25, 312.5*, 468.75*, 625*, 937.5* µg/mL 4 hour treatment, 20 hour harvest with activation (2% S9): 0*, 39.06, 78.13, 156.25*, 312.5*, 468.75*, 625* µg/mL</p> <p>Experiment 2 24 hour treatment, 0 hour harvest without activation: 0*, 9.77, 19.53*, 39.06*, 58.6*, 78.13, 156.25 µg/mL 4 hour treatment, 20 hour harvest with activation (1% S9): 0*, 78.13, 156.25*, 312.5*, 625*, 937.5, 1250 µg/mL * = Dose levels selected for metaphase analysis.</p> <p>Two hours prior to harvest the spindle inhibitor, Colcemid, was added to each culture to obtain a final concentration of 0.1 µg/mL. Slides were prepared using Giemsa stain. Two slides/treatment group were evaluated. 200 metaphase cells (100 per culture) were scored. Chromosomes were counted for each cell. Chromosome aberrations were recorded. The percent of aberrant cells and the frequency of aberration (%) per treatment group were determined. A positive response was recorded for a particular treatment if the percentage of cells with aberrations, excluding gaps, markedly exceeded that seen in the concurrent control, either with or without a clear dose response. For modest increases in aberration frequency a dose response relationship was generally required and appropriate statistical tests were applied.</p>
<u>Results</u>	Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes.
Remarks	<p>In the pretest toxicity assay a precipitate of the test material was observed in parallel blood free cultures at the end of exposure at ≥1250 µg/mL under all exposure conditions. In exposed cultures metaphase cells were present up to 625 and 1250 µg/mL in the 4/20 hour exposure groups in the presence and absence of metabolic activation. However, the frequency of metaphase cells was markedly reduced at 1250 µg/mL with metabolic activation. The maximum dose with metaphases present in the 24-hour continuous exposure was 312.5 µg/mL, where a markedly reduced frequency of metaphase cells was observed. Toxicity was observed under all exposure conditions.</p> <p>In Experiment 1, a dose related inhibition of mitotic index was observed. Mitotic inhibition was 45% at 625 µg/mL in the non-activated 4-hour exposure group. 82% mitotic inhibition was observed at 937.5 µg/mL, which was</p>

	<p>considered to be to toxic for metaphase analysis. With metabolic activation 29% mitotic inhibition was observed at the highest dose level tested (625 µg/mL). The doses selected for the analysis of chromosome aberrations were 156.25, 312.5, 468.75, 625 and, 937.5 µg/mL. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant.</p> <p>In Experiment 2, a dose related inhibition of mitotic index was observed. Mitotic inhibition was 68% at 58.6 µg/mL in the absence of metabolic activation. With metabolic activation the reduction in mitotic index was less clearly dose-related, with a plateau effect observed between 156.25 and 625µg/mL and with a mitotic inhibition of 35% observed at 156.25 µg/mL. The doses selected for the analysis of chromosome aberrations were 19.53, 39.06, 58.6, 78.13, 156.25, 312.5, 625µg/mL. The percentage of cells with structural or numerical aberrations in the test article groups was not significantly increased above the solvent control at any dose level. The percentage of structurally damaged cells in the positive control group was statistically significant.</p> <p>Positive and vehicle control group responses were as expected.</p>
<u>Conclusions</u>	Under the conditions of this study the test material was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes in the presences and absence of a liver metabolizing system at dose levels that induced acceptable levels of toxicity.
<u>Data Quality</u>	Reliable without restriction (Klimisch Code)
<u>References</u>	Chromosome Aberration Test in Human Lymphocytes in Vitro SafePharm Study 1666/0078
<u>Other</u>	Updated: 12/13/05